Analysis of the January 26, 1998 Performance Evaluation HIV-1 Antibody Testing Results Reported to the Centers for Disease Control and Prevention (CDC) by Laboratories Participating in the Model Performance Evaluation Program

This report is an analysis of results provided to the Centers for Disease Control and Prevention (CDC) by laboratories participating in the Model Performance Evaluation Program (MPEP) after they tested the human immunodeficiency virus type 1 (HIV-1) performance evaluation samples shipped to them January 26, 1998. Testing results were reported by 770 (90.2%) of 854 laboratories that were sent sample panels. One laboratory tested samples sent to them in the August, 1997 MPEP HIV antibody survey rather than the samples sent in the current survey. Additionally, result booklets were received from 2 laboratories more than three weeks after the cut-off date. Test results from these three laboratories are not included in the analysis.

Samples used in the MPEP surveys are undiluted, defibrinated plasma obtained from individual donors who are HIV-1 antibody-positive or HIV-1 antibody negative. The HIV-1 antibody-positive donor samples are heat treated. Before shipment, the CDC tested each donor sample with four HIV-1 and two HIV-1/HIV-2 enzyme immunoassay (EIA) kits licensed by the Food and Drug Administration (FDA). Supplemental testing was performed with three FDA-licensed HIV-1 Western blot (WB) kits and one HIV-2 WB kit. Donor samples were not tested by CDC with any HIV-1 indirect immunofluorescence (IIF) test.

The CDC sample reactivity shown in Figures 1, 5, 6, 7, 8, 9, and 10 is listed as negative or positive and was determined after composite EIA and WB testing with FDA-licensed kits and by using the WB interpretive criteria of the Association of State and Territorial Public Health Laboratory Directors/Centers for Disease Control (ASTPHLD/CDC) (MMWR 1989; 38, S-7: 1-7). The ASTPHLD/CDC WB interpretive criteria is the same criteria published in the package insert for all FDA-licensed HIV-1 WB test kits. In preshipment testing performed by CDC, the HIV-1 antibody strongly positive donor samples (Donors 17 and 18) were EIA repeatedly reactive with all of the HIV-1 and HIV-1/HIV-2 EIA kits and WB reactive with all HIV-1 FDA-licensed WB kits used by CDC. The negative donor samples (Donors 15 and 16) were EIA repeatedly non-reactive and demonstrated no bands with any FDA-licensed HIV-1 WB kit.

Donor samples 1-14, obtained from individual donors recently infected with HIV-1, were HIV-1 antibody weak-positive and demonstrated variable EIA and WB antibody reactivity with the FDA-licensed EIA and WB kits used for testing. Testing information for sequential serum samples from donors 1-14 demonstrated factors consistent with seroconversion such as a positive p24 antigen test, rising HIV-1 antibody titers in both lysate-based and recombinant antigen EIA tests with S/C ratios increasing as much as 10-fold between two bleeds, and WB reactivity changing from nonreactive (no bands) to reactive with the presence of antibody to p24 and gp120 and/or gp160 between bleeds.

Figure 1 shows the cumulative frequency of test result interpretations reported by participating laboratories, arranged according to sample reactivity, for the EIA, WB, and IIF methods. Of the 1,449 EIA interpretations reported for HIV-1 antibody-negative samples, 4 (0.28%) were incorrectly reported as reactive. False-negative EIA interpretations were reported for 14 (0.48%) of the 2,909 interpretations reported for the antibody-positive samples. One HIV-1 seroconversion sample (Donor 5) accounted for 5 (35.7%) of the 14 false-negative EIA interpretations reported. Of 256 WB interpretations reported for the HIV-1 antibody-negative samples, one false-reactive WB interpretation and eight indeterminate WB interpretation were reported. Among the 1,075 WB interpretations reported for the HIV-1 antibody-positive samples, there were no false-negative and 188 (17.5%) indeterminate interpretations. The weakly-reactive donor samples (Donors 1-14) accounted for all of the indeterminate WB

interpretations reported for the HIV-1 antibody-positive samples. Among the 47 IIF interpretations reported for HIV-1 antibody-negative samples, there were no false-positive or indeterminate interpretations reported. Of the 156 IIF interpretations reported for antibody-positive samples, there were 7 (4.5%) indeterminate and 5 (3.2%) false-negative interpretations. All false-negative and indeterminate IIF interpretations were reported for the HIV-1 antibody weak-positive seroconversion samples.

The types of laboratories that reported results to CDC are shown in Figure 2. Each laboratory type is listed, by decreasing frequency, for each of the test methods.

The combinations of test methods used by the laboratories and the frequency of use are shown in Figure 3. Most laboratories performed only EIA (60.7%), while some laboratories performed both EIA and supplemental tests (34.3%), and others (2.4%) performed only supplemental tests. There were 69 laboratories that performed other tests in addition to EIA, WB and IIF. Not represented in this figure are 31 laboratories that performed only tests other than EIA, WB, or IIF. The data for tests performed other than EIA, WB, or IIF are presented in Figure 10.

The types of kits used, by kit manufacturer, for the EIA, WB, and IIF methods are shown, by decreasing frequency, in Figure 4. For each test method, some laboratories indicated using test kits for which there was no unique glossary code provided in the survey report form and these responses have been grouped as "Other" manufacturer. Some "Other" kits reported as being used for EIA include Abbott HIV-1/HIV-2 3rd Generation PLUS, Murex ICE HIV 1.O.2 Detection, Innogenetics Innotest HIV-1/HIV-2, and Ortho Diagnostics HIV-1/HIV-2 Ab Capture EIA.

The results reported for the EIA, WB, and IIF methods, listed by kit manufacturer, for the positive and negative samples are shown in Figures 5, 6, and 7. Results reported by the participant laboratories reflect their testing performance using manufactured kits to evaluate MPEP samples and do not necessarily reflect an evaluation of these manufactured kits.

EIA Results

The four false-positive EIA interpretations were reported equally for Donor 15 and Donor 16 by laboratories using EIA kits from four different manufacturers (Figure 5).

Among the HIV-1 antibody-positive donor samples, there were 14 nonreactive EIA interpretations reported. All EIA false-negative interpretations were reported for the HIV-1 antibody weak-positive donor samples obtained from individuals during seroconversion (Donor numbers 1-14). False-negative interpretations were reported most often for Donor 5, 5 (35.7%) of 14, Donor 4, 3 (21.4%) of 14, and Donor 9, 3 (21.4%) of 14 results. Some laboratories reported initially reactive EIA results but nonreactive repeat EIA results for these seroconversion samples. The non-reactive EIA interpretations for HIV-1 antibody-positive donor samples were reported most often by laboratories using the Genetic Systems (Sanofi) HIV-1 LAV EIA kit, 7 (50%) of the 14 false-negative interpretations.

WB Results

Of the 770 laboratories reporting test results in this survey, only 270 (35.1%) performed WB testing. There were 8 indeterminate and one false-positive WB interpretations reported (Figure 6) for the HIV-1 antibody-negative samples (Donors 15 and 16). The indeterminate WB interpretations were reported equally for Donor 15 and Donor 16 and

were all reported by laboratories using the Cambridge Biotech (BioMerieux) WB kit. The laboratories reporting these indeterminate interpretations reported the presence of only p24, p51 or non-viral bands. The one laboratory reporting the detection of p24, p31, gp41, p66, and gp120/160 and a false-positive WB result for one of the Donor 15 samples in their panel reported no bands present and a negative WB result for the duplicate Donor 15 sample in the panel.

All of the indeterminate WB interpretations were reported for samples from the 14 HIV-1 infected, seroconverting donors (Donors 1-14). Indeterminate WB interpretations were reported most often for Donor 11, 39 (69.6%) of 56 interpretations; Donor 9, 58 (60.4%) of 96 interpretations; Donor 4, 15 (53.6%) of 28 interpretations; Donor 5, 12 (41.4%) of 29 interpretations; and Donor 8, 44 (38.6%) of 114 interpretations. Indeterminate WB interpretations for the seroconversion samples were reported by laboratories using WB kits provided by six different manufacturers. Among the FDA-licensed WB kits, the greatest frequency of indeterminate WB interpretations was reported by laboratories using a WB kit manufactured by Epitope/Organon Teknika,
88 (27.1%) of 325 interpretations (Figure 6).

Indeterminate interpretations reported for Donor samples 1-14 most often resulted from non-detection of antibody to envelope (env) antigens (e.g., gp41, gp160) or detection of env-antibody reactivity resulting in bands with less than the required intensity. The WB bands (of greater than or equal to 1+ intensity) for these donor samples, as determined in preshipment testing by CDC with three FDA-licensed WB test kits, are shown in Table 2.

Of the 270 laboratories reporting WB test results, 244 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria was used by 204 (83.6%) of these 244 laboratories. Some laboratories continue to indicate they use the WB interpretive guidelines described by the manufacturer of the WB kit they use and apparently are not aware that the WB interpretive guidelines published by the FDA- licensed WB kit manufacturers are identical to the ASTPHLD/CDC HIV-1 WB interpretive criteria. Five laboratories using the WB kit manufactured by BioRad indicated they were using interpretive criteria different from that recommended by the kit manufacturer as approved by the FDA.

WB Band Patterns

The protein band patterns for the major viral proteins, as reported by participant laboratories for each donor sample, are shown in Figure 8. The WB results include the testing of EIA-nonreactive donor samples, which most laboratories do not normally include in their algorithm of routine testing. The frequency of a reported band is listed above the column. The number of band pattern reports is listed in the far right column. This figure **does not** include WB bands reported as 'W', indicating intensity less than that of the designated band of the weak positive control provided in the WB kit nor does it include bands of greater than 1+ intensity reported for p15, p17, p51, p55, or p66.

Donors 15 and 16, HIV-1 antibody-negative donors, did not demonstrate antibodies to any of the HIV-1 viral-specific proteins or non-viral proteins in CDC preshipment testing with three FDA-licensed HIV-1 WB kits.

For the HIV-1 antibody strong-positive samples (Donors 17 and 18), laboratories had no difficulty in detecting antibodies to gag, pol, and env antigens with any HIV-1 or HIV-1/HIV-2 WB kit used. The donor material obtained from HIV-1 infected individuals during seroconversion, Donors 1-14, appeared to cause more difficulty. Most of the indeterminate WB interpretations reported for the seroconversion samples resulted from the laboratory failing to detect antibody to viral envelope antigen and, infrequently, to gag antigen in these donor samples. These findings are consistent with the CDC WB test results as shown in Table 2 of the results report accompanying this analysis.

IIF Results

No false-positive or indeterminate IIF interpretations were reported for the HIV-1 antibody-negative donor samples (Figure 7). Among the 156 IIF interpretations reported for the HIV-1 antibody-positive samples, 5 (3.2%) false-negative and 7 (4.5%) indeterminate interpretations were reported. No indeterminate or false negative interpretations were reported for the HIV-1 antibody strong-positive samples (Donors 17 and 18). For the seroconversion samples (Donors 1 - 14), false-negative and indeterminate interpretations were reported most frequently for Donor 5, 4 (36.4%) of 11 interpretations, and Donor 12, 5 (22.7%) of 22 interpretations.

Fluorescence Intensity Patterns

The IIF intensity patterns for HIV-1 infected cells, as reported by participating laboratories, are shown in Figure 9. The frequency of reports for fluorescence intensity patterns is listed in the far right column. A scoring of fluorescence intensity is not required for interpretation of seroreactivity with the FDA-licensed Waldheim Fluorognost HIV-1 IFA kit; therefore, some laboratories provided interpretation, but did not show fluorescent intensity. Data from these laboratories were included in Figures 1 and 7, but cannot be included in Figure 9.
No fluorescence intensity was reported for either of the HIV-1 antibody-negative samples (Donors 15 and 16). All laboratories reported 3+ or greater fluorescence for the HIV-1 antibody strongly-positive samples (Donors 17 and 18) with all commercial, noncommercial, and in-house IIF kits used. The IIF intensity reported for the weak-positive samples (Donors 1-14) frequently was greater than 1+, but occasionally no fluorescence (antibody) was reported for HIV-1 infected cells.

Other Tests Performed

Figure 10 provides information on the test results and interpretations provided by laboratories that do tests in addition to or other than microtiter-format EIA, WB or IIF. The first graphic of this figure shows manufacturers of the "Other" types of tests and frequency of use. The rest of this figure shows the results reported by laboratories after testing the HIV-1 antibody-negative and antibody-positive samples in this shipment. Thirty-one (31%) of the 100 laboratories reporting results of “Other” types of tests did not report results of EIA, WB or IIF tests. The procedures used by 59 (59%) of these 100 laboratories can be described as "rapid" microfiltration EIA procedures (e.g., SUDS HIV-1, Testpack HIV-1/HIV-2, MultiSpot HIV-1/HIV-2, and HIV-Spot HIV 1+2). These tests are generally provided as kits that use microparticles, such as latex, coated with purified lysate, synthetic, or recombinant HIV-1, and sometimes HIV-2 antigens. Fourteen laboratories tested samples using a gelatin particle agglutination test (Fujirebio Serodia HIV) and two laboratories used a latex agglutination test (Cambridge Biotech/BioMerieux Capillus). Results of “Line or Strip Immunoassay” tests such as Liatek (Organon Teknika), INNO-LIA (Innogenetics) and RIBA (Chiron) were appropriately reported on the “Other Test” results form by six laboratories.

Of the 100 laboratories reporting results on the form for “Other” types of tests, 51 are laboratories within the United States. Of these 51 laboratories, 50 reported results using the FDA-approved Murex SUDS HIV-1 test.

Among the 197 final interpretations reported for HIV-1 antibody-negative samples (Donors 15 and 16) tested by procedures other than EIA, WB, and IIF, two of the four false-positive interpretations were reported by one laboratory using the Cambridge Biotech Capillus agglutination test. All the indeterminate interpretations were reported by two laboratories using the Fujirebio Serodia HIV test. All four of the false-positive interpretations and two of the indeterminate interpretations were reported for Donor 16.

Among the 413 interpretations reported for the HIV-1 antibody-positive samples tested by procedures other than EIA, WB, or IIF, there were three false-negative interpretations and two indeterminate interpretations. False-negative and indeterminate interpretations were reported only for the seroconversion samples (Donors 1-14), and were reported most frequently, 1 (7.7%) of 13 reports for Donor 4, and 2 (7.7%) of 26 reports for Donor 13.

Quality Control Testing

Information was sought on the use of quality control (QC) samples **other than the controls provided in various test kits**. Positive and negative samples included in manufactured kits are internal kit control material used to validate the test run, calculate test run cut-off values, and may not validate the analytic testing process which may include testing problems such as faulty pipettors, inadequate incubation conditions, or kit lot sensitivity. Most laboratories completing the QC section of the form adhered to the instructions pertaining to this section and described only external QC samples used in their HIV testing procedures.

Of the 720 laboratories that reported EIA test results, only 403 (56.1%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 403 laboratories, 239 (59.3%) used samples obtained commercially, 145 (36%) used QC samples from in-house sources, and 12 (2.98%) used QC material from both commercial and in-house sources. Seven laboratories (1.7%) did not indicate the source of their external QC samples. The majority indicated the use of either weak-positive or weak-positive and negative serum/plasma with each set or run of EIA plates.

Of the 270 laboratories reporting WB test results, only 74 (27.4%) laboratories described their external QC samples. Of these 74 laboratories, 49 (66.2%) used samples prepared in-house, 25 (33.8%) used QC samples obtained commercially, and 3 (4.1%) used QC material from both commercial and in-house sources. Most laboratories used at least a weak-positive serum/plasma and included this sample in each set/run of WB strips.

Of the 39 laboratories reporting IIF results, only 12 (30.8%) used IIF external QC samples. Of these, 11 (91.7%) used samples from in-house sources and 1 (8.3%) used QC samples obtained commercially. The majority indicated that QC samples were included with each set/run of slides.

Of the 100 laboratories reporting results of tests other than EIA, WB or IIF, only 23 (23%) used external QC samples. Of these, 16 (69.6%) used samples from in-house sources and the majority indicated that a strong-positive QC sample was included with each run or at least with each new kit lot.

Conclusion

Most participant laboratories performed well in testing the HIV-1 donor samples in this shipment. No laboratories reported false-negative WB results and only a few laboratories reported false-negative EIA (0.48%) or false-negative IIF (3.2%) results for the HIV-1 antibody-positive samples (Donor numbers 1-14 and 17-18). Only rarely were false-positive EIA (0.28%) or false-positive WB (0.39%) results reported for samples that CDC tested and found negative for HIV-1 antibody in both EIA and WB tests (Donors 15 and 16).

The following information regarding overall analytic performance, analytic sensitivity, and analytic specificity is determined from the results reported by laboratories testing performance evaluation samples and is not intended to reflect the actual sensitivity and specificity of the manufactured test kits. For this survey, the overall EIA analytic sensitivity and specificity was 99.5% and 99.7%, respectively. When indeterminate and reactive WB interpretations are combined, the WB analytic sensitivity was 100%. If indeterminate interpretations are considered incorrect for

HIV-1 antibody-negative samples, the WB analytic specificity was 96.5%. When indeterminate and reactive IIF interpretations are combined for the HIV-1 antibody-positive samples, the IIF analytic sensitivity was 92.3%; the IIF analytic specificity was 100% for this survey. The analytic sensitivity and specificity of the test procedures other than EIA, WB, and IIF vary greatly, depending on which test results are analyzed (Figure 10). If indeterminate interpretations for the HIV-1 antibody-positive samples are combined with reactive interpretations, the overall analytic performance for laboratories testing these performance evaluation samples by EIA, WB, and IIF procedures was 99.6%, 99.3%, and 97.5% respectively.